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How Do Bacteria Decide Where to Divide?

Minireview

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When the time comes to undergo cytokinesis, the predivision cell must first decide where to initiate septal ingrowth. In rod-shaped bacteria such as Escherichia coli and Bacillus subtilis, the new septum is normally placed equidistant from the two cell poles. However, this is not invariant, and under certain conditions the cell places the division site at other specified positions along the long axis of the cell. These observations imply that cellular mechanisms exist that can process one-dimensional topological information and select specific sites along the long axis of the cell for the initiation of cytokinesis.

Two stages can be distinguished in the site selection process. First, the cell identifies and begins to differentiate the potential division site at midcell. Second, when the time comes to initiate septum formation, the cell selects the new site at midcell in preference to other potential division sites that are located elsewhere in the cell.

Division Site Selection during Symmetric Cell Division

The answer to how the cell first identifies the midcell site is still unclear (Woldringh et al., 1991; Cook and Rothfield, 1994). However, a considerable amount is known about the second stage in the site selection process, in which cells use the midcell site in preference to two other potential division sites that are located adjacent to the cell poles. Evidence that cells contain potential division sites at the poles has come primarily from studies of minicell mutants of E. coli (Adler et al., 1967). In these mutants, septation frequently occurs adjacent to one of the two cell poles, giving rise to small spherical cells ("minicells") that lack chromosomal DNA. Because the poles are derived from division sites that were located at midcell during a previous division event, the ability to form polar septa suggests that elements of the division machinery that are still competent to support cytokinesis remain at the ends of the daughter cells after septation and cell separation have been completed (Teather et al., 1974).

Reuse of the residual division sites at the poles gives rise to chromosomeless minicells. Therefore, their division potential must be suppressed, and this must be done without inactivating the potential division site that exists at midcell. Studies of the minicell genetic locus have indicated that this is accomplished by the cooperative action of a division inhibitor and a topological specificity factor that are encoded by the *minC*, *minD*, and *minE* genes (de Boer et al., 1989). Homologs of the E. coli *min* genes exist in species as diverse as Bacillus (discussed below) and Cyanobacteria (Kotani et al., 1995). The E. coli *min* gene products appear to function in the following way. First, the MinC and MinD proteins seem to act in concert to form a nonspecific division inhibitor that is capable of blocking septation at all potential division sites (Figure 1a). This follows from the observation that division is completely blocked when *minC* and *minD* are expressed in the absence of *minE*. Second, the MinE protein imparts topological specificity to the system so that the MinCD inhibitor blocks division at the polar division sites without interfering with septation at the midcell site (Figure 1b). Consistent with the predictions of this model, loss of either MinC or MinD leads to minicell formation, as expected if suppression of the polar sites is mediated by the MinCD division inhibitor, whereas loss of MinE leads to formation of nonseptate filaments.

The MinCD division inhibitor acts by interfering with the function of the essential cell division protein FtsZ. Overexpression of MinCD prevents formation of the FtsZ ring at midcell that is thought to be the initial step in cytokinesis (Bi and Lutkenhaus, 1993). Conversely, MinCD function can be counteracted by increasing FtsZ concentration (Bi and Lutkenhaus, 1990; de Boer et al., 1990).

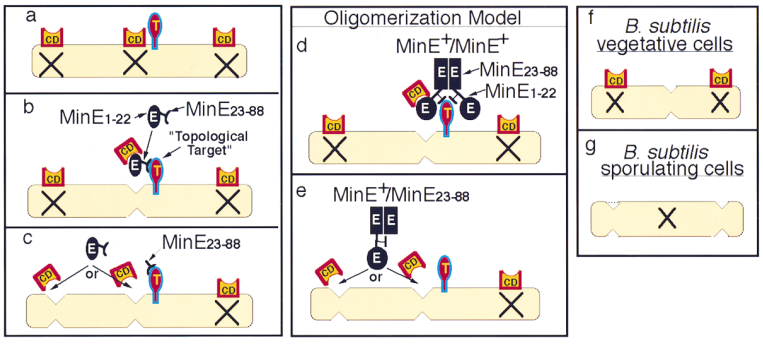

Figure 1. Models for Min Function

(a–c) Independent domain model. The topological sensor domain resides in MinE23-88, and the anti-MinCD domain is located in MinE1-22.

(a) Expression of *minCD* in the absence of MinE.

(b) Expression of *minC*, *minD*, and *minE* from the chromosomal *minCDE* locus.

(c) Expression of *minE23-88* in cells expressing *minC*, *minD*, and *minE* from the chromosomal *minCDE* locus.

(d and e) Oligomerization model. Oligomerization leads to formation of a topological sensor domain within the MinE1-22 region.

(d) Expression of *minCDE* in wild-type cells ($MinE^+/MinE^+$ homooligomers are illustrated as a dimer).

(e) Expression of *minE23-88* in cells expressing *minC*, *minD*, and *minE* from the chromosomal *minCDE* locus, leading to formation of $MinE^+$/MinE23-88 heterooligomers.

(f and g) Division site selection in B. subtilis during vegetative growth (f) and during sporogenesis (g). Division sites are activated at both poles although only one is usually used for septum formation (Ryter, 1964; Arigoni et al., 1995). T, topological target.

MinE carries out two functions. First, MinE is an antagonist of the MinCD division inhibitor since it prevents the filamentation that otherwise occurs when *minCD* is expressed. Second, MinE is a topological specificity factor since it gives site specificity to the division inhibitor, restricting its activity to the unwanted sites at the cell poles. Recent studies indicate that these two functions reside in different domains of the 88 amino acid MinE protein (Pichoff et al., 1995; Zhao et al., 1995). The ability of MinE to counteract the MinCD division inhibitor resides within a short N-terminal domain (MinE1-22) that is sufficient to prevent the filamentation that occurs when *minC* and *minD* are expressed in the absence of MinE.

The topological specificity function of MinE requires a domain within the C-terminal region of the protein (MinE23-81) since the ability of MinE to prevent minicelling in the presence of wild-type levels of MinCD is lost when C-terminal deletions extend past amino acid 81 (Zhao et al., 1995). The suggested role for the MinE23-81 domain in imparting topological specificity is supported by the finding that the MinE23-88 fragment affects the placement of the division septum even when separated from the N-terminal domain that is required to counteract the MinCD division inhibitor. Thus, MinE23-88 induces minicelling when expressed in wild-type cells in the presence of normal levels of MinC, MinD, and MinE (Figure 1c).

These results are consistent with a model in which the N-terminal domain of MinE acts as an antagonist of the MinCD division inhibitor, whereas the C-terminal region acts, directly or indirectly, as a topological sensor, capable of distinguishing the potential division site at midcell from the potential division sites at the cell poles.

We speculate that a target molecule, the topological target in Figure 1, marks the midcell site as being different from the poles. The high affinity of the topological sensor domain of MinE for this target would then lead to the preferential localization of MinE to the midcell site (Figure 1b), where the the N-terminal domain prevents the MinCD division inhibitor from blocking septation. Because MinE would be sequestered at midcell, it would not be available to interfere with the action of the division inhibitor at the cell poles. As a result, polar septation would be prevented and the normal division pattern would occur.

This model predicts that MinE is present at a limiting concentration within the cell. This view is supported by the observation that increasing the cellular concentration of MinE from the wild-type level of 200 molecules per cell to 400 molecules per cell leads to minicelling in wild-type cells (Zhao et al., 1995). In this situation, the MinE concentration presumably exceeds the level needed to saturate the topological target. The excess MinE would then be free to counteract the MinCD division inhibitor at the poles, leading to the minicell phenotype.

It has been suggested that the topological target may be located at the cell poles rather than midcell (Pichoff et al., 1995). In this view, MinE would act only as a pilot protein, directing the MinCD division inhibitor to the poles. Although this model cannot be excluded, it does not easily explain the observation that suppression of the polar division sites by the MinCD division inhibitor is lost when the cellular concentration of MinE is increased, as shown by induction of a minicelling phenotype (Zhao et al., 1995).

It is not yet known whether the MinE23-81 region that is required for topological specificity is itself the topological sensor domain. If this were correct, the induction of minicelling when MinE23-88 is expressed at high levels in wild-type cells would reflect a competition for the topological target between the MinE23-88 peptide and the wild-type copy of MinE (Figure 1c). This would free MinE molecules to counteract the MinCD inhibitor at the polar sites, leading to the minicell phenotype.

It is also possible that the MinE23-81 region does not interact directly with the topological target but instead acts only as an oligomerization motif (Figure 1d). In this model, oligomerization of MinE generates a topological sensor domain elsewhere in the protein through interactions between the N-terminal domains of adjacent MinE monomers. The induction of minicelling when the MinE23-88 fragment is expressed in wild-type cells would reflect the formation of mixed oligomers between the complete MinE protein and the MinE23-88 fragment (Figure 1e). The heterooligomers would lack the topological sensor domain but would still be capable of counteracting the MinCD division inhibitor at cell poles or midcell. There is evidence that MinE does exist as an oligomer and that the C-terminal region of the protein is required for oligomerization (Pichoff et al., 1995). At this point, it is not possible to choose between these two models for the domain structure of MinE.

The cellular locations of MinC, MinD, and MinE at wild-type levels of expression have not yet been established (the task is made difficult by their low abundance), and it is not known whether the Min proteins act directly or via intermediary proteins.

Identification of the topological target molecule is the next major challenge since all models require that the system be able to distinguish between central and polar sites. It would not be surprising if the target proved to be a component of the normal division apparatus whose properties (for example, affinity for MinE) were altered during the division process. In this way, the new division site at midcell could have a high affinity for the MinE topological sensor whereas the previously used division sites at the poles would not.

Division Site Selection during Asymmetric Cell Division

In E. coli, the Min system prevents septation at polar division sites. This control is never circumvented except in the pathologic cases in which mutations or experimental manipulations lead to loss of function of the MinCD division inhibitor or overproduction of MinE. In contrast, in B. subtilis and other spore-forming bacteria, polar sites are used to support septum formation during sporogenesis.

When B. subtilis grows vegetatively, septation is restricted to midcell. However, under conditions of nutrient deprivation or of interference with guanine nucleotide synthesis, the site of septation switches to one of

the two cell poles. Formation of the polar septum is followed by a series of differentiation events that ultimately lead to release of a mature spore. The spore septum is formed at approximately the same relative position as the polar septa in minicell-producing bacteria, raising the possibility that the Min system that normally regulates use of polar division sites in E. coli has its counterpart in Bacillus and suggesting that the use of polar sites during spore formation might be accomplished by additional layers of regulation superimposed upon this system. It is therefore of interest that minicell-producing mutations have been isolated and homologs of the E. coli *minC* and *minD* genes have been identified in B. subtilis.

Mutations at either of two genetic loci in B. subtilis *(divIVA* and *divIVB*) are associated with a minicelling phenotype (Reeve et al., 1973). The chromosomal region that includes the *divIVB* gene contains two open reading frames (now called *minC* and *minD*) that code for proteins with significant homology to the E. coli *minC* and *minD* gene products (Lee and Price, 1993; Levin et al., 1992; Varley and Stewart, 1992). Strong evidence for the functional similarity of the B. subtilis and E. coli Min proteins comes from the observation that point mutations or insertion mutations in the B. subtilis *minC* or *minD* gene lead to a minicelling phenotype (Lee and Price, 1993; Levin et al., 1992; Varley and Stewart, 1992). Further, introduction into E. coli of plasmids that contain the B. subtilis *minC* and *minD* genes leads to minicelling in the E. coli host (Varley and Stewart, 1992). This suggests that one or both of the B. subtilis proteins may compete with their E. coli counterpart for binding to a target required for inhibition of polar septation events, with the B. subtilis protein(s) being able to bind to the E. coli target but unable to suppress its septation potential.

Thus far, a B. subtilis counterpart of the E. coli *minE* gene has not been found. If the parallelism between the B. subtilis and E. coli systems is valid, a B. subtilis *MinE* homolog should exist. Since MinE is responsible for the ability of the E. coli Min system to discriminate between polar and midcell division sites, identification and characterization of the putative *minE* gene of B. subtilis is of crucial importance if one speculates that the B. subtilis Min proteins play a role in the polar placement of the spore septum. An obvious place to search for the missing *minE* gene is in *divIVA*, the second B. subtilis locus in which mutation leads to minicelling.

Let us examine the possibility that the sites that are used in formation of the spore septum in B. subtilis are equivalent to the polar sites that are suppressed by the Min system in E. coli. According to this way of thinking, during vegetative growth, B. subtilis resembles E. coli, with MinC and MinD working in concert, probably in cooperation with a MinE protein yet to be found, to suppress the use of the polar sites without interfering with use of the potential division site at midcell (Figure 1f). However, if the Min paradigm is to be applied to formation of the prespore septum, it must deal with the fact that when sporulation is induced, the topological specificity of the system is reversed. The polar sites are released from suppression to permit formation of the prespore septum, and septation at the midcell site in the mother cell is now suppressed (Figures 1f and 1g).

Although other models are possible, all of the septation-related phenomena could be explained by scenarios that are based on the E. coli Min paradigm in which a topological specificity factor determines whether a MinCD division inhibitor blocks division at midcell or at the cell poles. A simple model predicates the existence of two MinE proteins, with different topological properties. These could represent different primary gene products or could result from posttranslational modifications that change the topological sensor properties of a single *minE* gene product. The vegetative MinE would counteract the action of the MinCD division inhibitor at midcell but not at the cell poles, whereas the sporulation-specific MinE would counteract the action of the division inhibitor at the polar site but would allow the division inhibitor to block septation at midcell.

This model predicts that the block to septation at the midcell site during sporogenesis will not occur in the absence of the *minC* and *minD* gene products, although the polar septation event and subsequent stages of spore morphogenesis will proceed normally. The availability of knockout mutations in *minC* and *minD*, which have been shown not to interfere with sporogenesis, should make it possible to test this prediction.

It is interesting that a mutation in the second B. subtilis minicell locus, *divIVA*, leads both to minicelling and to the formation of long nonseptate filaments (Reeve et al., 1973). This combination might be expected if the *divIVA* mutation induced cells to undergo the characteristic shift in topological specificity of septal placement that occurs at a very early stage in sporogenesis, without progression to subsequent stages of the sporulation pathway (Figure 1g). The release of the polar division block would give rise to minicell formation, whereas the inhibition of septation at the midcell site would lead to filamentation. There is presently no published information on whether the *divIVA-1* mutant is, in fact, blocked in sporulation, as predicted by this model.

Although the Min proteins are unlikely to be solely responsible for the shift in division site placement that occurs during sporogenesis, at a minimum one would predict the occurrence of a sporulation-specific gene product that acted to reverse the MinCD-mediated suppression of polar septation events.

Selection of the Plane of Division

Following the establishment of the division site, the cell must specify the plane of division. In globular or cuboid bacteria, several possible orientations of the division septum are compatible with the production of two daughter cells of the same size and shape. In these cases, the pattern of division plane selection determines the architecture of the arrays of progeny cells that often remain associated with each other after division is completed. In chain-forming organisms, the plane of division is approximately the same in each division cycle (-x-x-x-). However, in many bacterial species, the orientation of the division septum alternates between planes that lie at right angles to each other. Interestingly, this includes E. coli *rodA* mutant cells that grow as spheres (Donachie et al., 1995). In these cases, one might expect that the x, y, and z planes would be selected at random since they are topologically equivalent. Instead, there is

a rigid selection of certain orientations. In some species, such as Lampropedia (Murray, 1984), only two of the three possible planes are used, in an alternating sequence (-x-y-x-y-). This leads to formation of large square planar arrays of cells. In contrast, in organisms such as Sarcinae (Canale-Parola, 1970), cells are organized into three-dimensional cubical 8-celled packets that can only result from the ordered and sequential use of all three of the division planes (-x-y-z-). In each of these examples, the choice of the division plane in each generation determines the two-dimensional or three-dimensional organization of the multicellular arrays of progeny cells that are formed. Essentially nothing has been done to study the mechanisms responsible for these fascinating simple systems of multicellular differentiation.

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